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THE DESIGN AND EVALUATION OF
IN SITU BIORESTORATION METHODS
FOR THE TREATMENT OF SLUDGES AND
SOILS AT WASTE DISPOSAL SITES

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ABSTRACT

In situ methods for treatment of waste sludges hold great promise for efficient remediation of sludge at waste disposal sites. The remediation of the diverse and complex sludges from the O.E. MacDougall Waste Disposal Site, near Brockville, Ontario, by current conventional techniques would be very costly. Natural bioremediation techniques were tested in the laboratory, using six representative soils and sludges obtained from the MacDougall site. These techniques require a minimal amount of sludge manipulation, so their use at the site is viewed as a cost-effective method of site cleanup.

Liquid industrial wastes (including solvents and waste oils), sewage sludge and septic tank wastes were disposed of in waste lagoons at the MacDougall site. Six samples of soil and sludge were collected for characterization of the organic chemistry of the materials. Four samples were found to contain varying concentrations of aliphatic and aromatic hydrocarbons typical of petroleum products and chlorinated hydrocarbons typical of solvents.

Standard plate counts and culture tube techniques were performed to estimate the number of aerobic, anaerobic (denitrifiers, sulphate-reducers and methanogenic) and sulphate-reducing bacteria present in each material type. All sludge and soil samples contained viable bacteria of the three kinds tested for. Typically, aerobic bacteria were the most abundant, while the sulphate-reducers were the least abundant. Anaerobic bacteria were more abundant in the more contaminated sludges than in the cleaner soils.

The diverse organic chemistry and nature of the microbial population allowed the testing of a number of remedial environments in the laboratory. Bioremediation experiments employed sealed glass bottles containing soil or sludge and distilled water. Various chemical additions were made to selected bottles to establish aerobic, methane-oxidizing, denitrifying, sulphate-reducing or methanogenic conditions within the bottles. At various times, duplicate bottles were analyzed for target aromatics (benzene, toluene, xylenes, trimethylbenzene, cumene, naphthalene) and chlorinated hydrocarbon (tetrachloroethene, trichloroethene) concentrations.

The aromatic hydrocarbons were most easily broken down under aerobic conditions, but also degraded anaerobically upon the addition of nitrate and/or sulphate. Tetrachloroethene and trichloroethene were easily broken down to dichloroethene under anaerobic conditions. These results are promising, since they suggest that the natural degradative processes, perhaps acting in sequence and with some stimulation, may provide significant remediation of the sludges at the MacDougall site.

1.0 INTRODUCTION

A common waste form is the wet solid termed 'sludge'. Sludge may come from municipal waste treatment plants and various industrial sources. Often, liquid waste forms are mixed with wet solids, which produces a potentially more hazardous waste. Disposal of such wastes could produce considerable environmental damage, especially to surface waters and groundwaters. Harmful chemicals may leave the sludge as non-aqueous phase liquids (NAPLs) and as dissolved constituents in infiltrating water. These sources of groundwater contamination must be either rendered non-toxic or isolated from the hydrosphere. Typical sludge treatments include landfarming to promote aerobic biotransformations and volatilization of toxic organics, physical/chemical treatment such as incineration, and hydraulic isolation in secure landfills. Current alternatives are costly and/or imperfect. Additional, innovative treatments are required to provide more cost-effective and efficient sludge remediation.

On-site treatment holds the greatest prospect for cost-effective remediation. Utilization of natural remedial mechanisms, especially biotransformation, may also be more cost-effective than capital- and energy-intensive physical/chemical treatments, such as incineration or chemical oxidation. An example of such a treatment scheme is the landfarming of petroleum-industry sludges, where the biodegradation and volatilization of organics and the immobilization of many metals is encouraged. Whereas this treatment is successful for certain contaminants, it is ineffective for others. Thus, for sludges containing a broad spectrum of toxic organics and metals, no single natural remediation scheme is likely to be satisfactory.

Various industrial and municipal sludges were disposed of at the O.E. MacDougall Waste Disposal Site near Brockville, Ontario. The remediation of these diverse and complex sludges by current techniques would be very costly (Monenco, 1986). Thus, there is a need to develop in situ treatments for sludges with complex chemistries. Because these techniques require a minimal amount of waste manipulation, their use at waste disposal sites is viewed as a cost-effective method of site cleanup.

The objective of this research was to evaluate the potential for the natural biotransformation of selected organic contaminants in order to remediate the various industrial and municipal sludges found at the MacDougall Waste Disposal Site.

Laboratory experiments were conducted to assess biotransformation by in situ microbial communities under a number of biogeochemical environments. The results from these experiments may be used to design in situ sludge treatments where the desired biogeochemical environment within the sludge may be achieved with minimal manipulation to promote the desired microbial responses. The results from this research should have a broad application to waste disposal site remediation in general, since the occurrence of complex sludges is not likely unique to this site.

2.0 SCOPE OF THE RESEARCH

The research undertaken was a laboratory study which first concentrated on characterizing the microbial communities and the organic chemistry of six representative soils and sludges from the MacDougall site.

The second phase of research consisted of two bioremediation experiments in which the fate of selected target organics was monitored in a variety of biogeochemical environments. The results from these experiments may be incorporated into in situ bioremediation techniques at the MacDougall site.

3.0 SITE DESCRIPTION AND BACKGROUND

The O.E. MacDougall Waste Disposal Site is located approximately 5 km northwest of Brockville, Ontario. The following background information has been obtained from a report by Monenco (1986). The site is comprised of about 33 hectares, of which 4 were used for waste disposal activities from 1962 to 1981. Liquid industrial wastes (including solvents and waste oils), sewage sludge and septic tank wastes in unknown amounts were disposed of in waste lagoons, which were excavated into sandy overburden and bermed with dried sludge residue. Spreading fields were utilized for the disposal of sewage since at least 1979. The lagoons and spreading fields are currently covered with 0.3 to 0.6 m of sandy soil. Domestic refuse, building debris, scrap metal, hospital kitchen wastes and oil spill cleanup wastes were also disposed of at this site. The locations of the lagoons and spreading fields are shown in Figure 3.1.

At present, the MacDougall site is utilized as a waste transfer station. Liquid industrial wastes are collected from local industries and temporarily stored prior to transport to

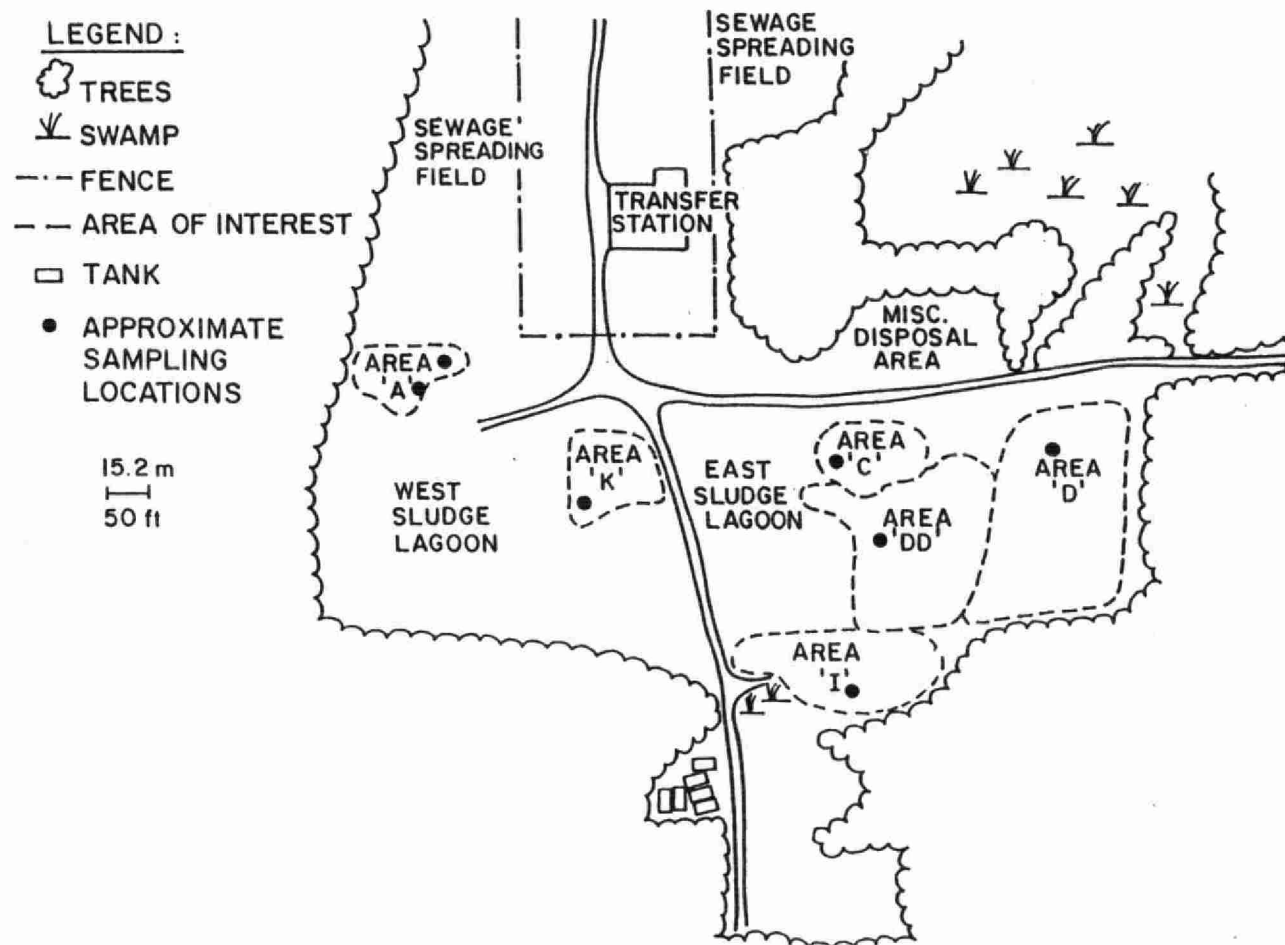


FIGURE 3.1. MCDOUGALL WASTE DISPOSAL SITE MAP (AFTER MOE, 1984).

other sites for disposal. No waste materials have been disposed of at this site since November 1981.

During the summer of 1984, the Ontario Ministry of the Environment (MOE) conducted an evaluation of the waste materials disposed of at the MacDougall site. This on-site investigation was conducted in response to hydrogeological studies which reported the occurrence of two contaminant plumes emanating from the site. Monoaromatic hydrocarbons and chlorinated aliphatics were identified in the groundwater.

During the MOE's investigation, trenches were dug to bedrock by backhoe. Soil and sludge samples were collected and analyzed for acetone, monoaromatics (benzene, toluene, ethyl benzene and xylenes) and selected chlorinated aliphatics (1,1,1-trichloroethane, tetrachloroethene and trichloroethene) by gas chromatography. Analytical results from this soil survey indicated the presence of significant concentrations of monoaromatics and chlorinated aliphatics in sludges and soils of Areas A, C and D (Figure 3.1) and trace levels of chlorinated aliphatics in sludge from Area K.

Further investigation was conducted on-site in September 1985 by Monenco Consultants, for the purpose of assessing the extent of soil contamination around the site perimeter. Soil and sludge samples were analyzed for the same target compounds analyzed for in 1984. Traces of the target compounds were found in soil samples obtained from the sewage spreading field, and in samples obtained northwest and southeast of the lagoons. Significant concentrations of the target compounds were found in soils and sludges obtained from the east and west sludge lagoons and from an industrial sludge lagoon located in the vicinity of Area I.

4.0 CHARACTERIZATION OF THE SLUDGES AND SOILS

4.1 Sample Collection

Samples of soil and sludge were collected in October 1987 from Areas A, C, D, DD, I and K, as indicated on Figure 3.1. Samples were collected from Areas A, C, D and K because these were identified by the MOE (1984) as being the most contaminated. Area DD appeared to be a separate lagoon not identified in the MOE report, so samples were collected here also. Although significant levels of contamination were not previously

found in samples from Area I (MOE, 1984), the Monenco report (1986) identified an industrial sludge lagoon in this area with significant levels of toluene and xylenes.

Twelve fresh soil and sludge samples were collected by spade at the seven locations indicated in Figure 3.1. All samples except those obtained in Area C were obtained by hand digging to the desired depth interval in an undisturbed area. Area C samples were obtained by cleaving back a trench wall (previously dug by backhoe in 1984) to expose fresh material. Table 4.1 describes the samples and the depth intervals from which they were obtained. Samples were carefully collected to minimize disturbance and aeration of the material. The samples were collected in 250 mL screw cap glass jars, stored on ice, and transported to the Organic Geochemistry Laboratory at the University of Waterloo. Samples collected from Areas A, C and I were dark brown to black medium-grained sandy material, while samples collected from Areas D, DD and K were black sludges with a strong odour of digested sewage.

4.2 Characterization of Organic Chemistry

The soils and sludges were screened to identify as many organic compounds as possible, since this had not previously been done for soils and sludges at the MacDougall site (Monenco, 1986). The screening included quantification of aromatic and chlorinated hydrocarbons (halocarbons), and identification of volatile organic compounds present in the soils and sludges.

Volatile organic compounds present in the soils/sludges were identified using gas chromatography and mass spectrometry (GC/MS). One to two grams of fresh sample material was placed into a purge vessel to which 20 mL of organic-free distilled water was added. The vessel was shaken, allowed to stand and then purged with helium gas. The helium was analyzed by GC/MS with approximate detection limits of 0.5 ug/g. Individual compounds identified by GC/MS are listed in Appendix 1. The data are summarized in the last column of Table 4.2.

Routine aromatic and halocarbon analyses were performed using micro-extraction techniques with gas chromatography. The compounds analyzed included benzene, toluene, ethyl benzene, xylenes, cumene, 1,2,4-trimethylbenzene, naphthalene, 1,1,1-trichloroethane (TCA), trichloroethene (TCE), tetrachloroethene (PCE), chloroform and

TABLE 4.1: SUMMARY OF SLUDGE AND SOIL SAMPLES COLLECTED FROM THE MACDOUGALL SITE, OCTOBER 1987

Area	Sample No.	Depth Interval (m)	Sample Description
A	A1	0.30	dark grey, green fine sand, faint sewage odour
	A2	0.46	
	A3	0.51	
	A11	0.20	
	A22	0.20-0.30	
C	C1	0.56	sampled trench at MOE location C3; black oily sludge
	C2	0.58-0.69	
D	D1	0.51	sewage sludge; no overlying sand layer
DD	DD1	0.30	oily sludge; no overlying layer
I	I1	0.91	sampled trench at MOE location I1; dark brown fine sand; no sludge
	I2	1.22	
K	K1	0.91	sewage sludge overlain by 0.90 m of sand

chlorobenzene. This list of compounds includes those analyzed by the MOE in previous studies. One to two grams of sample material was transferred to an 18 mL glass hypovial which was then topped up with organic-free distilled water and capped with a teflon-faced silicon septa and aluminum crimp seal. After shaking, a small amount of water was withdrawn and replaced, via syringe, with pentane. The pentane effectively concentrated the organics and was analyzed by two gas chromatographs for aromatics and chlorinated aliphatics separately. The results of the analyses are presented in Table 4.2.

Samples obtained from Areas C, D, DD and K have significant concentrations of aromatics (29 to 540 ug/g), while only the sludge samples from Areas C and D were found to contain significant concentrations of halocarbons (8 to 1,317 ug/g). Tetrachloroethene (PCE) and trichloroethene (TCE) were the most abundant halocarbons identified, with concentrations up to 404 and 913 ug/g, respectively. Trichloroethane concentrations were quite low, with the maximum concentration (0.004 ug/g) occurring in C1 sludge. Sample K1 contained 0.02 and 0.38 ug/g of chloroform or chlorobenzene, respectively.

No volatiles were identified in the samples from Area A. However, samples from Areas C, D, DD and K were found to contain volatile organics in addition to those quantified by the GC scans. Dichloroethene (DCE) was identified in most samples containing significant TCE and PCE. PCE and TCE are likely to have originated as solvent materials, while DCE is thought to be a biotransformation product resulting from the dechlorination of PCE and TCE (Parsons et al., 1985).

Methyl- and ethyl-substituted benzenes were identified in samples from Areas D, DD and K. These samples also contain significant concentrations of other lighter aromatics, indicating the sludges to be petroleum-rich. The sludge from Area D also contains chlorinated solvents.

Substituted cycloalkanes, branched ketones and branched long chain aliphatics were identified in Samples C2, DD1 and C1, respectively. Cycloalkanes and branched aliphatics probably represent oily wastes, while the ketones may be biodegradation products of the original hydrocarbons.

TABLE 4.2: SUMMARY OF ANALYTICAL RESULTS - SOILS AND SLUDGES FROM THE MACDOUGALL SITE, 1987

Sample No.	Aromatic Hydrocarbons (ug/g)							Naphthalene	Chlorinated Hydrocarbons (ug/g)			Other Compounds Identified by GC/MS
	Benzene	Toluene	Ethyl Benzene	P/M-Xylene	O-Xylene	Cumene	1,2,4-TMB		TCA	TCE	PCE	
A1	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	ND	NA
A2	ND	ND	ND	ND	ND	ND	ND	ND	0.003	0.02	ND	NA
A3	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND	NA
A11	ND	0.03	ND	ND	ND	ND	ND	ND	ND	0.005	ND	ND
A22	ND	0.01	ND	ND	ND	ND	ND	ND	ND	0.002	ND	ND
C1	3.27	ND	ND	ND	ND	2.69	2.92	20.32	0.004	1.06	6.72	DCE, branched aliphatics with GT 10 carbons
C2	0.37	ND	ND	ND	ND	ND	ND	0.92	ND	0.10	0.43	Substituted cycloalkanes
D1	5.10	285.89	28.19	115.27	38.91	0.98	50.57	16.15	ND	913.02	404.76	DCE, substituted benzenes
DD1	4.28	37.10	5.51	20.84	4.87	ND	12.62	14.97	0.002	0.16	0.06	DCE, substituted benzenes, branched ketones
I1	NA	NA	NA	NA	NA	NA	NA	NA	ND	0.01	ND	NA
I2	0.07	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND	NA
K1*	1.88	19.59	5.61	22.51	21.21	2.51	95.49	23.42	ND	0.04	0.03	DCE, substituted benzenes

* Sample K1 also had chloroform at 0.02 ug/g and chlorobenzene at 0.38 ug/g.

TMB - trimethylbenzene
DCE - dichloroethene
TCA - trichloroethane
TCE - trichloroethene
PCE - tetrachloroethene
NA - not analyzed

The analytical results for Areas C, D and K presented in Table 4.2 generally agree with those presented by the MOE (1984). Results from Areas A and I are not in agreement because the MOE sampled sludges from these areas, whereas these sludges could not be located during the 1987 sampling events and apparently less contaminated soils were obtained instead.

In summary, soils from Areas A and I were not significantly contaminated. Sludges from Areas DD and K are petroleum sludges, while those from Areas D and C are complex sludges containing components of both petroleum products and chlorinated solvents.

4.3 Characterization of Microbial Populations

Bioremediation of sludges is dependent upon the number and types of microorganisms present within the sludge or soil material. Standard plate counts and culture tube techniques were performed to estimate the number of viable aerobic, anaerobic and sulphate-reducing bacteria present in each soil or sludge type. Table 4.3 presents the results of the bacterial enumerations.

All samples contained viable bacteria of the three kinds tested for. Generally, the aerobes were the most and the sulphate-reducers were the least abundant. Typical numbers of aerobic bacteria ranged from 10^6 to 10^9 per gram of sample; however, sample D1 was found to contain only 6.5×10^4 aerobes per gram. Sample D1 contained very high concentrations of aromatic and chlorinated hydrocarbons, so the lower number of aerobes may be due to the toxicity of these compounds. Anaerobic bacteria numbers ranged from 10^4 for uncontaminated soils to 10^6 for contaminated sludges. Uncontaminated soils and complex sludges contained up to 5×10^3 sulphate-reducers; however, the petroleum sludges contained significantly more sulphate-reducers with numbers up to 8×10^5 .

The soils and sludges of the MacDougall site were found to contain a wide variety of viable bacteria. Both aerobic and anaerobic bacteria were present, thus creating the potential for both aerobic and anaerobic bioremediation schemes.

TABLE 4.3: SUMMARY OF THE BACTERIA ENUMERATIONS

Sample No.	Sample Type	Depth Sampled (m)	Number of Bacteria		
			Aerobic (cfu/g) ¹	Anaerobic (cfu/g) ¹	Sulphate Reducers (M.P.N.) ²
A1	Uncontaminated Soil	0.30	1.29×10^6	5.28×10^4	4.9×10^2
A2		0.46	1.57×10^7	1.08×10^5	5.4×10^3
A3		0.51	5.9×10^5	1.64×10^4	3.5×10^3
I1	Uncontaminated Soil	0.91	1.46×10^7	7.10×10^4	4.9×10^2
I2		1.22	1.01×10^7	1.28×10^4	1.7×10^2
C1	Complex Sludge	0.56	1.76×10^8	7.50×10^6	1.7×10^3
C2		0.58-0.68	2.41×10^8	2.16×10^6	2.0×10^1
D1	Complex Sludge	0.51	6.46×10^4	3.14×10^6	2.0×10^3
DD1	Petroleum Sludge	0.30	8.25×10^8	3.46×10^6	7.9×10^5
K1	Petroleum Sludge	0.91	1.12×10^9	5.01×10^6	7.9×10^4

¹ colony forming units per gram of sample.² most probable number per gram of sample.

5.0 LABORATORY BIOREMEDIATION EXPERIMENTS

The diverse organic chemistry and nature of the microbial population within the sludges and soils allowed the testing of a number of remedial environments in the laboratory. The environments tested and the critical additions required to create that environment include:

- o aerobic (O_2 present or added),
- o aerobic, methane oxidizing (O_2 , CH_4 added),
- o anaerobic, sulphate reducing (O_2 excluded, SO_4 added),
- o anaerobic, denitrifying (O_2 excluded, NO_3 added), and
- o anaerobic, methane producing (methanogenic) (O_2 excluded).

Aromatic hydrocarbons are known to break down relatively rapidly under aerobic conditions (Dagley, 1984) and, to a lesser degree, under denitrifying (Zeyer *et al.*, 1986; Major *et al.*, 1988) and methanogenic conditions (Wilson and Rees, 1985). Chlorinated hydrocarbons are documented to be transformed by sequentially losing chlorine from the molecule. This process, referred to as "reductive dehalogenation" may occur in anaerobic environments (Parsons *et al.*, 1985) with or without biological intervention. Recently, aerobic methane-oxidizing bacteria were documented to biodegrade some chlorinated hydrocarbons (Wilson and Wilson, 1985). Thus, aerobic plus methane-oxidizing bacteria could remove both hydrocarbons and halocarbons.

The bioremediation experiments employed sealed glass bottles containing sludge or soil material and distilled water, which were incubated at room temperature. Various chemical additions (e.g., sodium nitrate, methane) were made to selected bottles to establish the desired remedial environment. At various times, duplicate bottles were analyzed for target organic concentrations.

Table 5.1 lists the aromatics and halocarbons which were selected for routine analyses. The basis for the selection of these compounds as "target" organics was that the compounds were:

- o present at significant concentrations in most soils or sludges,
- o listed in the Ontario Government's Regulation 309,

TABLE 5.1: TARGET ORGANIC COMPOUNDS FOR ROUTINE MONITORING OF
BIOREMEDIATION EXPERIMENTS

Aromatic Hydrocarbons

Benzene

Toluene

Ethyl Benzene

Xylenes

1,2,4-Trimethylbenzene

Cumene

Naphthalene

Chlorinated Hydrocarbons

Tetrachloroethene

Trichloroethene

1,1,1-Trichloroethane

- o water soluble and so of greatest potential mobility in groundwater, and
- o easily analyzed by existing GC techniques.

5.1 Experiment 1 - Aerobic and Methane-Oxidizing Conditions

Sludges containing high concentrations of aromatics were selected for evaluation of bioremediation under aerobic conditions. Those sludges containing halocarbons were also used for evaluation of methane-oxidizing conditions.

Individual 60 mL glass bottles for this experiment contained 3 g of sludge from either Area D, DD or K and 20 mL of deionized water. Ten mL of methane gas was added to the atmosphere of bottles containing sludge from Areas D and DD. The concentrations of oxygen, carbon dioxide, methane, aromatics and halocarbons were monitored over 147 days. One-half of the total number of bottles received 1 mL of a 10% sodium azide solution to inhibit bacterial action and thus provide a sterile control for the experiment.

Figure 5.1 shows the concentration of oxygen in types DD1 and K1 microcosms with time. Oxygen concentrations decreased in both sterile and active (i.e., non-sterile) microcosms; however, the losses were much greater for active microcosms. The losses in sterile microcosms are assumed to be due to imperfect sterilization and diffusion of oxygen out through the seal of the bottles, while the losses in active microcosms are assumed to be mostly due to the consumption of oxygen during biodegradation. Oxygen concentrations decreased within all active bottles from about 16% to less than 3% within 55 days. Pure oxygen was added to the microcosms on Day 88 in order to maintain aerobic conditions. By Day 147, the oxygen concentrations were again less than 3%. Oxygen losses in microcosms containing methane did not differ significantly from those without methane.

Figure 5.2 shows the concentrations of oxygen, methane and carbon dioxide in active type D1 microcosms. Methane concentrations did not decrease over 147 days, indicating methane oxidation had not occurred.

Carbon dioxide concentrations increased over time in both sterile and active microcosms, indicating that aerobic respiration had occurred. The accumulation of carbon dioxide was greater in active microcosms (13 to 18%) than in the sterile microcosms (up to 5%). Presumably, sterilization of the controls was incomplete.

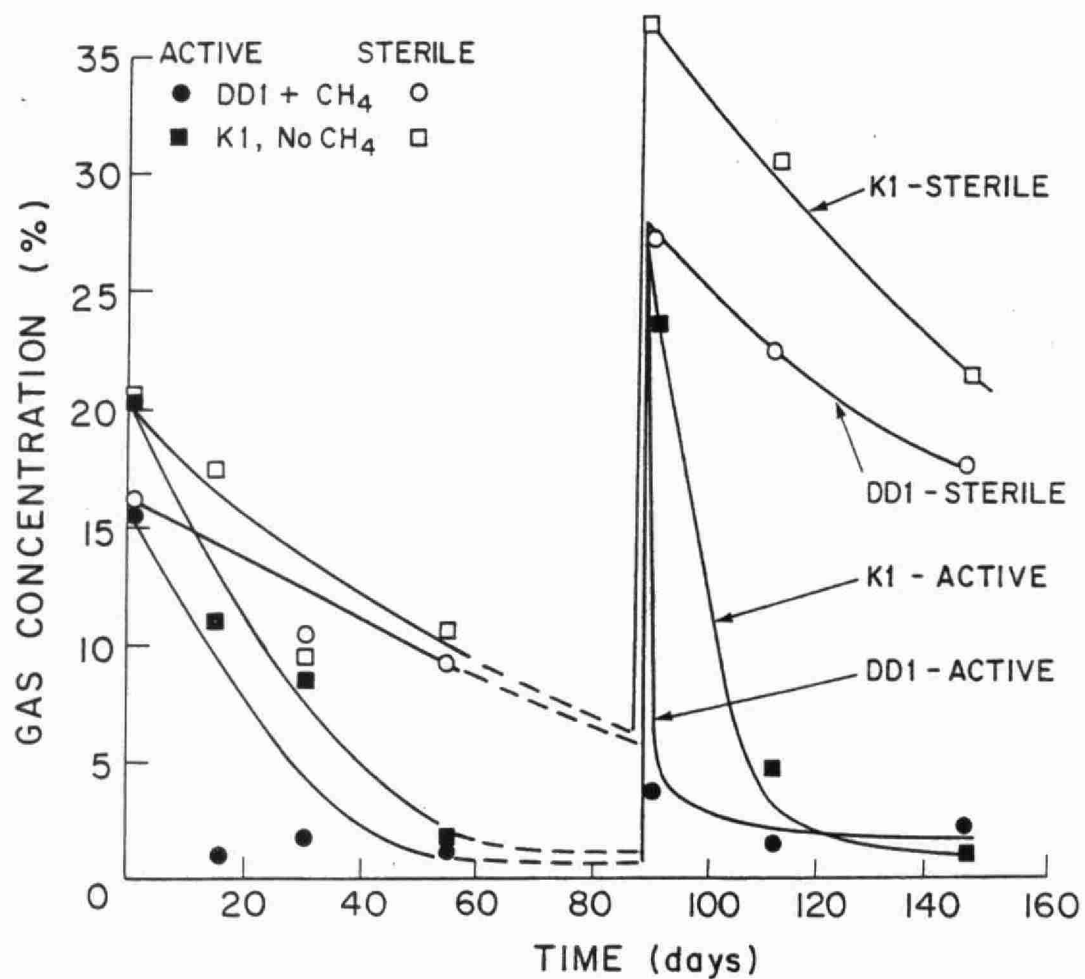


FIGURE 5.1. CONCENTRATION OF OXYGEN IN MICROCOSM HEADSPACE, EXPERIMENT 1.

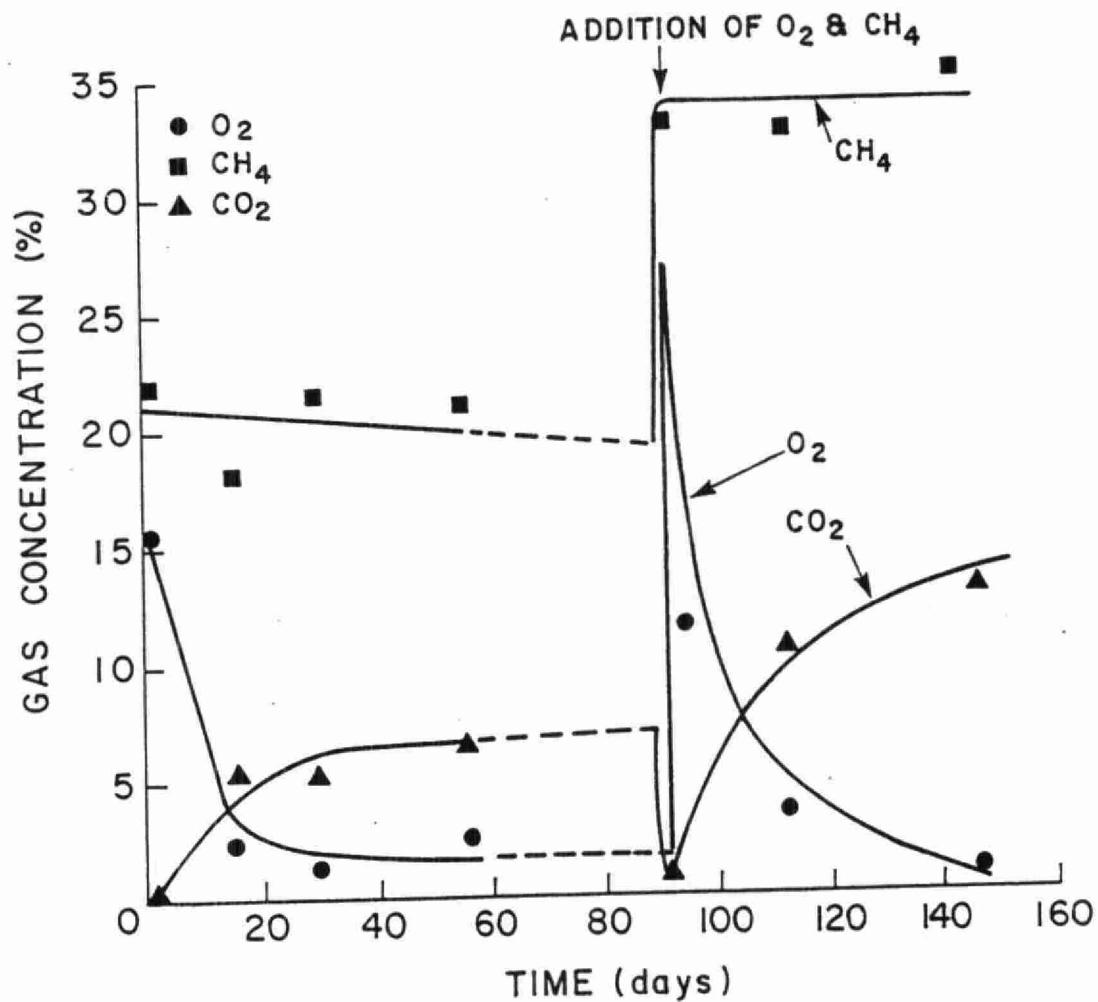


FIGURE 5.2. CONCENTRATION OF GASES IN TYPE D MICROCOSM HEADSPACE, EXPERIMENT 1.

The initial concentrations of target organics in active microcosms are presented in Table 5.2. Comparison of these data with the concentration data obtained during the screening of sludges (Table 4.2) indicates there to have been a substantial decrease in concentration between the time of screening in November 1987 and the initiation of the bioremediation experiment in January 1988. Concentrations decreased by over three orders of magnitude for halocarbons, by about one order of magnitude for benzene, toluene, ethyl benzene and xylenes, and by about 50% for trimethyl benzene, cumene and naphthalene. These losses are consistent with the relative volatilities of the compounds. Volatilization likely occurred during storage and handling during the experimental setup.

The concentration of aromatics in active type D1 and K1 microcosms decreased by about 50% within 147 days. Figure 5.3 is a plot of the aromatic concentration versus time for active and sterile microcosms containing D1 sludge. Losses were greatest when oxygen concentrations were high and the biodegradation rates slowed when oxygen became limiting. Active type DD1 microcosms do not show significant losses compared to the sterile controls. The initial aromatic concentration was much lower (less than 25 ug/g) than for type D1 and K1 microcosms, and perhaps this low concentration could not sustain the bacterial activity. The aromatic content was not reduced below about 25 ug/g in D1 or K1 microcosms, supporting the concept of a "threshold" or minimum concentration requirement.

Toluene shows the most significant concentration decrease of the aromatics. Benzene and o-xylene concentrations in active microcosms did not vary significantly from those of the sterile controls. No changes in either PCE or TCE concentrations occurred with time.

Aerobic conditions promoted the biodegradation of most aromatic compounds. Benzene, o-xylene and chlorinated hydrocarbons were recalcitrant. Methane oxidation did not occur and, therefore, is not likely to be a viable bioremediation technique for sludges at this site.

TABLE 5.2: INITIAL CONDITIONS FOR EXPERIMENT 1

Compound	Concentration ¹ (ug/g)		
	D1	DD1	K1
Benzene	0.6	0.6	0.9
Toluene	10.6	1.3	3.1
Ethyl Benzene	6.0	1.1	2.3
P/M-Xylene	19.5	2.1	1.5
O-Xylene	5.0	1.3	5.7
1,2,4-Trimethylbenzene	15.7	8.5	26.0
Cumene	1.1	0.3	1.3
Naphthalene	8.6	5.4	21.6
1,1,1-Trichloroethane	N.D.	N.D.	N.D.
Trichloroethene	0.6	N.D.	N.D.
Tetrachloroethene	1.6	N.D.	N.D.

¹ Concentrations shown are average of duplicate active microcosms.

N.D. - not detectable.

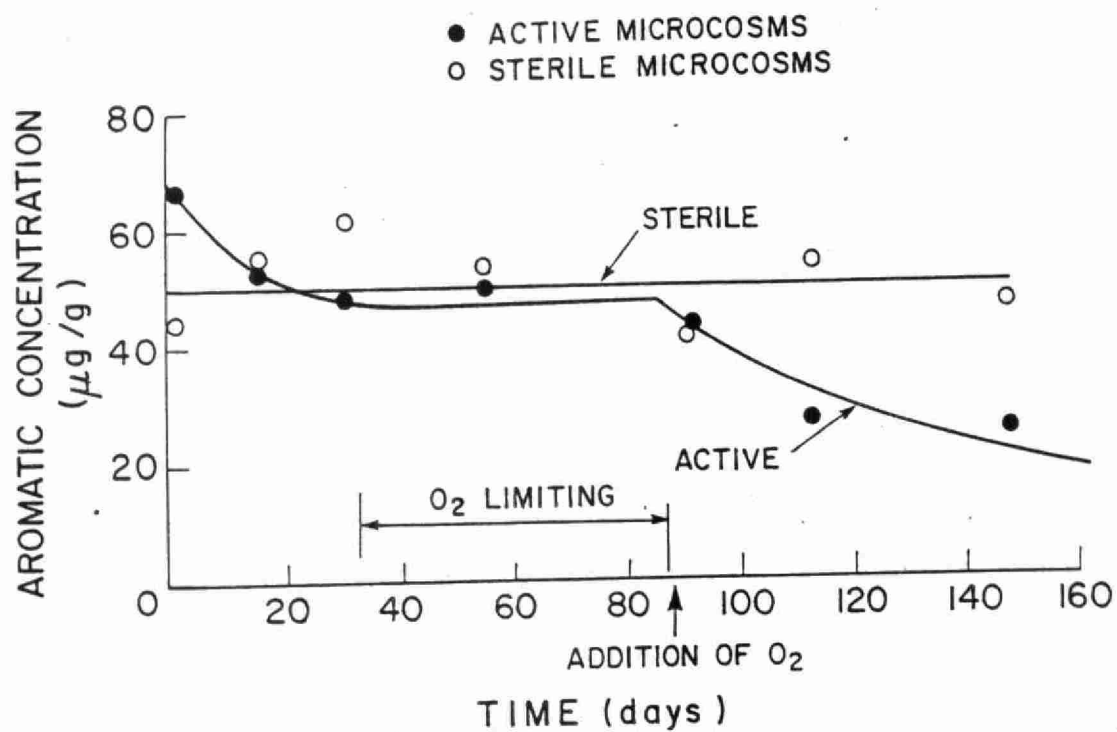


FIGURE 5.3. TOTAL AROMATIC HYDROCARBON CONCENTRATION IN TYPE D MICROCOSMS, EXPERIMENT 1.

5.2 Experiment 2 - Anaerobic - Denitrifying, Sulphate-Reducing and Methanogenic Conditions

Microcosms, set up in 18 mL glass hypovials, contained 1 g of sludge from either Area C, D, DD or K. The hypovials were filled with deionized water to which the target organics had been added to duplicate the concentrations obtained during screening (Table 4.2). This addition of organics was made because of the large volatile losses experienced for Experiment 1. Some of each microcosm type were amended with sodium nitrate to give a nitrogen concentration of about 70 mg/L. Some of the microcosms containing sludge from Areas C and DD were amended with sodium sulphate to give initial sulphate concentrations of about 200 mg/L. These microcosms were thought to be representative of denitrifying and sulphate-reducing environments, respectively. The remaining microcosms, to which no additions were made, were thought to represent methanogenic conditions. The experimental design is summarized in Table 5.3. The concentrations of target aromatics and halocarbons, sulphate and nitrate were monitored over 214 days.

Microcosms containing the most contaminated sludge (from Area 'D') with and without nitrate showed a decrease in PCE and TCE concentrations from 100 ug/g to less than 1 ug/g within 12 days. This decrease was accompanied by an increase in an unidentified compound over 24 days. A special qualitative analysis by GC, with GC/MS confirmation, indicated this compound to be *cis*-1,2-DCE. This compound remained relatively consistent over the remainder of the experiment.

The decrease in PCE and TCE and increase in DCE concentrations indicates that a reductive halogenation breakdown mechanism was likely responsible for the observed trends. The common end product of such a process is generally vinyl chloride, the most toxic of this chlorinated aliphatic group. Thus, the transformation of PCE and TCE may result in the production of a more unfavourable compound if the dehalogenation reaction proceeds beyond DCE. Unfortunately, it was not possible to analyze for vinyl chloride using the analytical methods employed for the target organics. However, DCE concentrations did not seem significantly reduced over 214 days, suggesting that further dechlorination to vinyl chloride may not have occurred in significant amounts.

Reductions of PCE and TCE was not detected in microcosms containing sludges or soils from Areas C, DD or K. PCE and TCE concentrations in these soils and sludges were

TABLE 5.3: EXPERIMENTAL DESIGN FOR ANAEROBIC TREATMENTS,
EXPERIMENT 2

Sludge Type	Amendments		
	Nitrate Only	Sulphate Only	No Addition
C1	X	X	X
D1	X		X
DD1	X	X	X
K1	X		X

approximately 0.5 ug/g. Similarly, further reduction of PCE and TCE was not detected after 12 days in D type microcosms when concentrations had been reduced to about 0.5 ug/g. It is likely that 0.5 ug/g represents a threshold concentration below which insufficient energy is gained from utilizing the reduction reaction. Thus, this may be the minimum concentration of PCE and TCE achievable by anaerobic processes.

Microcosms containing sludges from Areas K, DD and D show some reduction of the aromatic content. Decreases are most apparent in toluene concentrations, but trimethylbenzene and naphthalene concentrations are also decreased somewhat over time. Toluene decreases are most significant in microcosms amended with sulphate or nitrate (Figure 5.4).

Figure 5.5 is a plot showing nitrate concentrations over time for 'denitrifying microcosms'. Nitrate became depleted within 45 days of the onset of the experiment. Sodium nitrate was added, using aseptic techniques, on Days 48 and 116. Nitrate was most quickly utilized in microcosms containing D1, DD1 and K1 sludges. The reaction utilizing nitrate is likely to be denitrification.

Sulphate concentrations in 'sulphate-reducing microcosms' were reduced from 3.6 mg/g to 0.2 mg/g in less than 12 days in microcosms containing DD1 sludge. Additions of sodium sulphate were made on Days 48 and 116 with subsequent similar reductions afterwards. The microcosms containing C1 sludge did not show sulphate losses.

The lack of sulphate-reducing activity in C1 microcosms may be due to the lower number of sulphate-reducers present (Table 4.3) or the lack of a suitable substrate for the sulphate-reducing bacteria. The aromatic content of this sludge was quite low, initially, and may be insufficient to promote significant sulphate-reducer activity.

In summary, PCE and TCE were transformed by a reductive dehalogenation process resulting in the accumulation of cis-1,2-DCE. It is uncertain whether vinyl chloride was also produced during this process. The addition of nitrate and sulphate promoted the transformation of toluene, trimethylbenzene and naphthalene, but only when the initial aromatic concentration was greater than about 50 ug/g. Benzene, ethyl benzene, xylenes and cumene were not transformed.

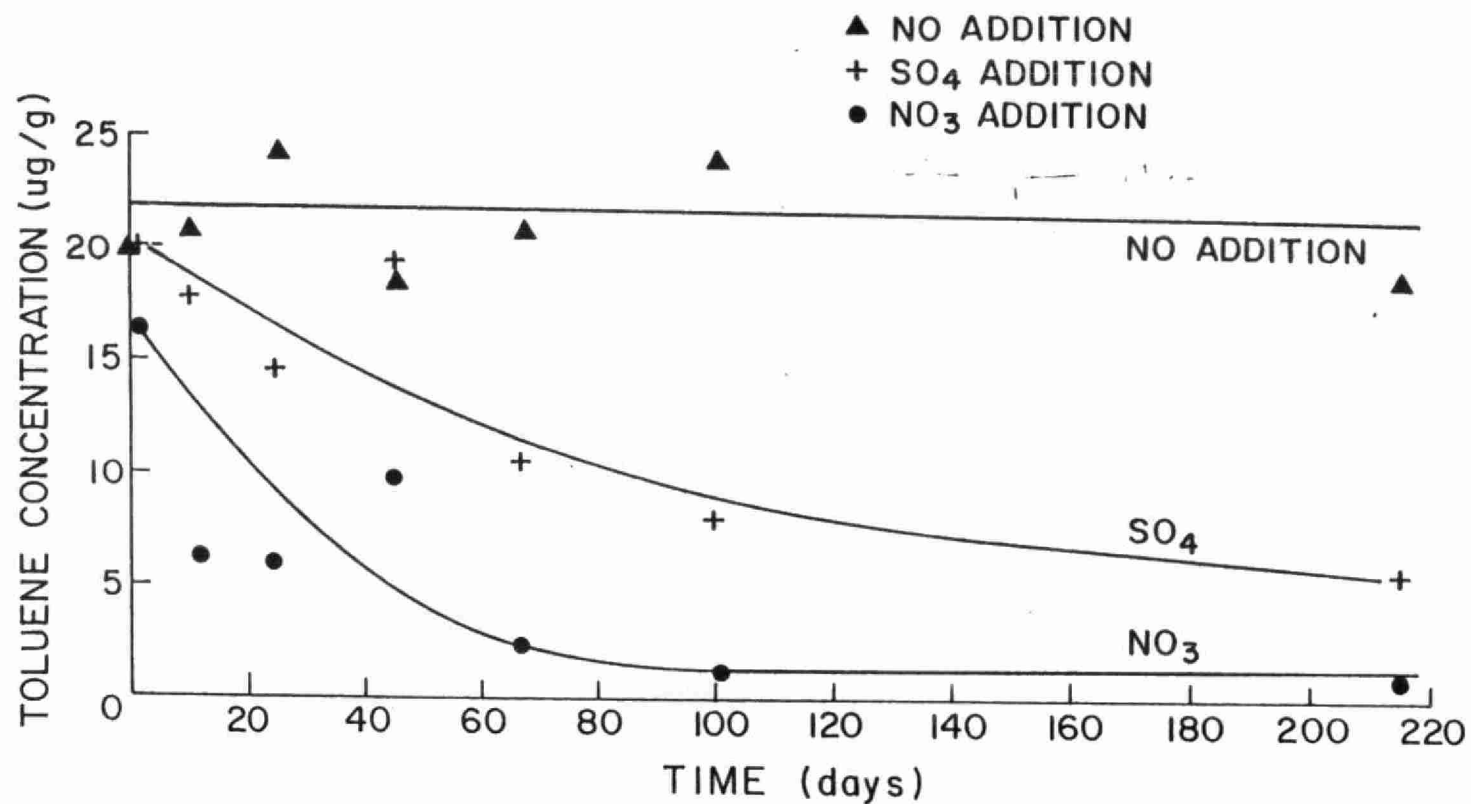


FIGURE 5.4. TOLUENE CONCENTRATION IN TYPE DD MICROCOSMS, EXPERIMENT 2.

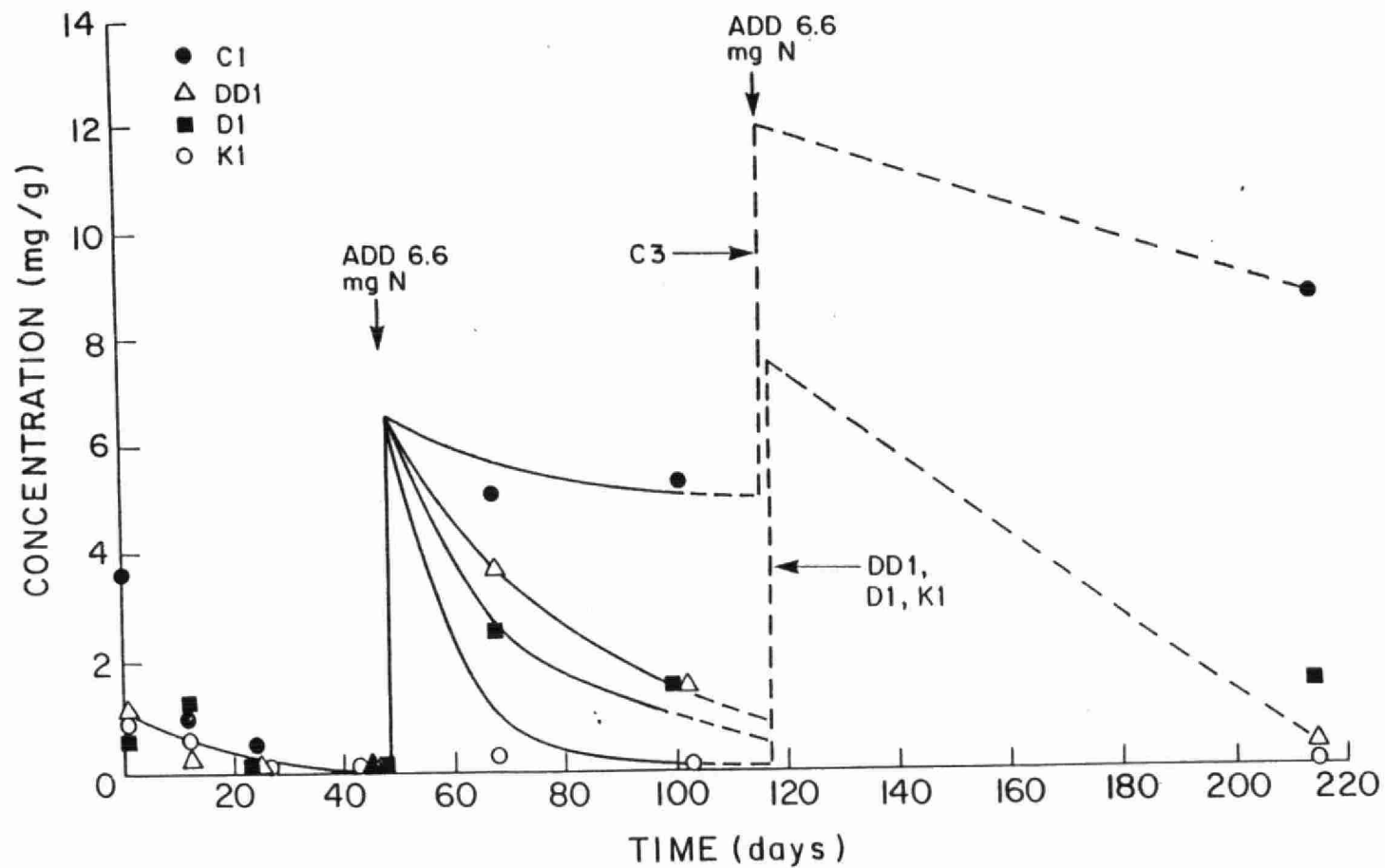


FIGURE 5.5. NITRATE CONCENTRATION IN DENITRIFYING MICROCOSMS, EXPERIMENT 2.

6.0 CONCLUSIONS

1. Sludges and soils obtained from the MacDougall site contain organic compounds typical of petroleum hydrocarbons and chlorinated solvents.
2. Contaminated sludges and soils from the MacDougall site contain a wide range of viable bacteria.
3. Aerobic conditions were the most favourable for the biodegradation of aromatic compounds, but did not promote halocarbon biodegradation.
4. Trichloroethene and tetrachloroethene were transformed under all anaerobic conditions.
5. The addition of nitrate and sulphate during anaerobic conditions promoted the degradation of some aromatics (toluene, trimethylbenzene and naphthalene).
6. Benzene and o-xylene were not transformed under any conditions.

7.0 RECOMMENDATIONS

1. Lab studies suggest that the natural anaerobic environment for many sludges/soils at the MacDougall site is conducive to dechlorination reactions removing some tetra- and tri-chloroethenes, but producing toxic and more-mobile di- and perhaps mono-chlorinated ethenes. Some cis-1,2-dichloroethene was apparently produced, while monochloroethene (vinyl chloride) was not determined. There is a chance that vinyl chloride could be produced naturally in at least some of the sludges/soils. It is recommended that contaminated groundwater be analyzed for these breakdown products as part of the ongoing site monitoring program. It is also recommended that their concentrations be determined in any additional lab or field tests.
2. Under anaerobic conditions, dechlorination reactions did not appear to be inhibited by nitrate and sulphate additions, while aromatic degradation was enhanced. Perhaps most aromatics could be biodegraded by denitrifiers and/or

sulphate reducers over longer times (greater than 300 days). If a single environment is to be selected for in situ bioremediation, the anaerobic environment, amended by nitrate and sulphate, is the most promising. Therefore, it is recommended that anaerobic conditions in subsequent tests be supplemented by the addition of both nitrate and sulphate as alternate electron acceptors. It is also recommended that anaerobic conditions, with nitrate and sulphate additions, be one of the remedial conditions evaluated in further tests.

3. No single microbial environment produced rapid biodegradation of both aromatics and chlorinated aliphatics. However, most organics showed some degradation potential in some microbial environments. It is recommended that subsequent lab and field studies investigate the use of a sequence of microbial environments for in situ sludge/soil reclamation.

Typically, many of the harmful products of anaerobic metabolism are readily degraded in aerobic environments. Therefore, if a sequence of microbial environments is to be established, the last environment should be aerobic. It is recommended that an anaerobic then aerobic sequence of microbial environments be tested for the potential degradation of organic contaminants of concern at this site. If aeration is used to create the aerobic conditions, the organics can be removed by both volatilization and aerobic biodegradation. In subsequent lab and field tests involving aeration, the loss of organic contaminants via volatilization and via biodegradation should both be determined.

4. Biodegradation rates may be enhanced by the addition of nutrients, such as phosphorus and reduced nitrogen compounds. It is recommended that subsequent tests evaluate the benefit of additions of such nutrients.
5. Some success in supporting and enhancing biodegradation rates has been demonstrated in the lab. Further tests should also be conducted under realistic but controlled field conditions. It is recommended that controlled in situ field tests use in situ columns, such as those developed by Dr. R.W. Gillham at Waterloo. Promising remedial techniques should also be tried in small pilot plots on-site before full-scale remediation is considered.

6. Organic compounds, in addition to target compounds, are present in sludges/soils at the site. Gross indicator parameters should be included in at least field tests in the future. These should include oil and grease to monitor higher-molecular-weight hydrocarbons and BOD or COD to keep track of the major organic substrates for the bacteria. Occasional GC/MS scans should be run to follow the progress of other organics at least qualitatively and to look for potentially harmful breakdown products.

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APPENDIX 1

**MacDougall Soils and Sludges
GC/MS Data**

APPENDIX 1: MACDOUGALL SOILS AND SLUDGES GC/MS DATA

Sample No.: A11
Internal Standard: PFT, 1.994 ug/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
PFT (int. std.)	6.571

Sample No.: A22
Internal Standard: PFT, 707.7 ng/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
PFT (int. std.)	6.575

Sample No.: C1
Internal Standard: PFT, 1,853 ng/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
DCE	3.039
PFT (int. std.)	6.613
PCE	7.401
Methyl tridecane	11.743
Methyl undecane	11.979
Dibutyl cyclopentane	12.397
Tert-butyl methyl aziridinone	13.020
(Cyclohexyl methyl)-meth-cyclohexane	13.455
Dimethyl nonane	14.762
Methyl propyl cyclohexane	15.007
Methyl hexadecane	15.284
Decahydro naphthalene	15.717
Dimethyl ethyl benzene	15.874, 16.728
Methyl tridecane	17.258
Decahydro methyl naphthalene	17.529
Hexyl heptadecane	17.823
Pentyl methyl oxiraneundecanoic acid	18.058
Propyl heptanol	18.107
Cyclohexyl octane	18.311
Methyl nonadecane	19.232
Dibromo dodecane	19.904

Sample No.: C2
Internal Standard: PFT, 1,724 ng/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
PFT (int. std.)	6.615
PCE	7.407
Trimethyl-methyl cyclohexane	12.400
Butyl propyl cyclopentane	15.371
Diethyl-methyl cyclohexane	16.438
Decahydro-methyl naphthalene	17.535
Cyclopentylethy cyclopentane	18.055
Ethyl-methyl-butenyl cyclohexane	19.269, 19.695, 19.743
Dibromo dodecane	19.910

Sample No.: D1
Internal Standard: PFT, 14 ug/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
DCE	3.071
TCE	4.508
Toluene	6.203
PFT (internal standard)	6.636
PCE	7.506
Ethyl benzene	9.175
Xylene	9.498, 10.259
Ethenyl benzene	10.161
Propyl benzene	12.346
Ethyl methyl benzene	12.627, 12.861, 13.228, 13.734
Propenyl benzene	13.842
Dimethyl decane	14.010
Dichlorobenzene	14.269
Ethyl methyl benzene	14.660
Methyl phenyl hexane	14.763
Methyl propyl benzene	15.640
Butyl benzene	15.788
Dimethyl ethyl benzene	15.882
Methyl propoyl benzene	16.131
Ethyl dimethyl benzene	16.481, 16.535, 16.741
Dimethyl decane	17.267
Tetramethyl benzene	17.712, 17.847, 18.835

Sample No.: DD1
Internal Standard: PFT, 2,811 ng/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
DCE	3.037
PFT (internal standard)	6.623
Cycloheptatriene	6.147
Ethyl benzene	9.158

Xylene	9.451, 10.223
Methyl ethyl benzene	12.628, 12.856, 13.235
Tetramethyl pentanone	12.970
Methyl heptanone	13.651
Trimethyl benzene	13.706, 14.656
Dimethyl decane	14.003
Methyl phenyl hexanone	14.763
Methyl propyl benzene	15.640
Ethyl dimethyl benzene	15.882, 16.477, 16.531, 16.470, 18.834, 17.847
Trimethyl nonyl benzene	16.126
Dimethyl decane	17.270
Tetramethyl benzene	17.714
Naphthalene	19.751

Sample No.: K1
Internal Standard: PFT, 5.36 ug/g

<u>Compound I.D.</u>	<u>Retention Time (min)</u>
1,1-DCE	2.991
Toluene	6.075
PFT (internal standard)	6.568
Ethyl benzene	9.093
Xylene	9.376, 10.166
Dimethyl decane	10.511, 13.952, 17.205
Propyl benzene	12.281
Ethyl methyl benzene	12.570, 13.168
Trimethyl benzene	12.798, 13.650, 14.597
Dichlorobenzene	14.204
Ethyl dimethyl benzene	14.706, 15.821, 16.421, 16.475, 16.683
Methyl (methylethyl) cyclohexanol	14.857
Methyl propyl benzene	15.580
Trimethylnonyl benzene	16.070
Tetramethyl benzene	17.654



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